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BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITU

DATE: _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT Fosmid clones from Lawrenceville

For hkcNQ5, see p. 72

Steve Dworetzky
Neuroscience
Bristol-Myers Squibb
5 Research Parkway
Wallingford,CT 06450

Steve Dworetzky,

In pINCy vector

(partial) hkcNQ5

Enclosed you will find a LB +Amp petri dish streaked with the clones 3447387, 4970006, 3532089, 1858095, and 3458089. These all originate from a single colony and are stored as glycerol stocks. If you have any questions, please do not hesitate to contact me.

chloride ch. chloride ch.

already have - Nach - Asic - like family

hkcNQ4 (partial)

Sincerely,

Tiziano DiPaolo

Tiziano DiPaolo
Applied Genomics
Core Sequencing Facility
Bristol-Myers Squibb
Lawrenceville,NJ
609-252-3991
dipaolot@bms.com

These clones have already been sequenced —

1858095 – we already had – Na Ch – Asic - like family

3447387, 3532089 – are chloride channels – give to Mark Thompson @ to work on —

4970006 – is a partial clone; hkcNQ5 potassium channels

3458089 – is a partial clone; hkcNQ4

Cont'd →

SIGNED Joanne Tynadka DATE

WITNESSED AND UNDERSTOOD BY: Puja Agarwal Billis DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

DATE: _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT hKCNQ5

See page 42 for 5'rapte clone info

EST — from Lawrenceville — 4790006 — partial —

[Fwd: Sequence Information] hKCNQ5

Subject: [Fwd: Sequence Information]
Date:
From: "Steven I Dworetzky" <dworetzs@bms.com> **Internal**
Organization: Bristol-Myers Squibb
To: "Trojnacki, Joanne T" <trojnacj@bms.com>

Clone: 4970006
Library: kidney epithelial transf embryo line, 293-EBNA, lg cDNA
Putative Annotation: KCNQ Family Member ??
Vector: pINCY
Sequence:

```
CCTTTTTTTTTTTTTTTTTTTTTTGAGATGGAGTCTCTCTTCTTCACCCAGACTCTGCC
TCAAAATAAATTATGGCACATACAGTAGGATAAAAACCAGTCAAAAGAGAAATTCTGTCT
CTAACAATAGCACCTCAGTACACTGTAGTACTGAGTTCTAGGCATCAATCATATATTAAC
TCATTAAACTCTGACAACAATCTTATGAGACTGGTATTACTAATATTCCCCCATTTAAAA
AATGGAGAAATTGTAATTTACCTAAGACCATTGGATAAATTGTTTTGAATGAATCTGAAT
TTAAGTAGTCTGACTCCAATGTCCACATGCTTTTAACCCTTACATGTGTTTAGTGACATG
GAAAGATATTCACGATATACTGAATGAAAAAGAGTATTGTTCAACAGCATTTTTTGAAAG
AAAATAAATTTATGTGCATCCTTTCACACTGTTCTTCTGGATTTCCTCCCACTTTCCCAT
CTTATCTCAAACTCAGATTAAAAATATACATACATACATATATACATATGTGTATAGGTA
GGTAATGCATACTTATATAACCATAGGCCAAAGCATGGGATAAAGGTTTTATACCAAGTT
TTTATTATGGTCATCCCTGGGAACAGGATTATGATTGGGCTTTCTTTATCTCTGTAGTGT
CTGCATACACATGTAGGACACGAAATAAGCATATAATACTTTGGGAAGGCCAGAGAAAAA
CACTCTGTCATTTGCTTTTAACCACGGACAGCAAACAAAAAACCTATTCTGACTGAAAAA
TTTCTGTGCCCAACATGAAGTGCTAAGAACAATTCTGACTGATCATCTCCAGTCTATTAA
ATCTATTGAATGTATTCTATGACTACCTAATGTTTCATATAAGTAAATAAATTACATTTG
GAGGAATATATCTGACCTACCTACTGGACACAAAATGATTTCATGATGCTGCCTAGTATT
GTGAATCATTCTTTTAGGTCCATAGCTTTTTAGCTACCTGATAGGCTATTACTAACCAAA
AAGAAGTTGGTCCCGTTCAGAAAGTAGGTGTCTTTAAAACCTAACTGAGCAACACAGAAT
TCATCCTATATCTATCCCATTCAAAAAAATTATAAATCCAACTTTCCCACTACTGAGACA
AAAGTCTTAAGGTGATCTTGAGCTTTGATCTCTCAATTATCAACTAGGGGCTGGGGGAAG
AATTAGATGTTTTCTCTGGCTAATACCATTACTATTCTACAAATAAATCCTAGACATTTA
ATGTATGTTAATGAGATAATCTGTTAGTACTTAACACAGAGTATAATTGTTCAAGAAATG
TTAGTTCTGTCCCCTCATCACAAGAAATGCTTATAATACAGAGAAATCTGAAGTCTCTCT
GTAGCTATACTGAAAATTCCTTAGGCTCAATTCATAAGCACTTTGAAGGCCAGGCTCATT
GGCTCACACCTATAATCCCAGTGCTTTGGGAGGCCAAGGCAGGAGGACTGCTTGAGTCCA
GGAGTTTGAGACCAGCCTGAGCAACATAGTGAGACCCCTGTCTCTACAATATTTTTAAAA
TTAGCTGGGCATGGTGGCATCTACCTGTAATTCTAGCTCCTCAAGAGGCTGAGGAGGGAG
GATCTGTAAAGTTGTTGGGGCTGCTGGCTTGGGTGCCGTATTTATTTGGTTTGCAATGGT
GTTGGTGGCTGCCACTGCTGAGCCATCGCTTTGACTAATTGGCACCTGTGTTGCTTGACT
GTGCATAGTAGGGCTAAGCGCGTAGAAAGTCTGGGCACTGAACTCATTTGGCGTCAGAAT
GAACTGCAGGCCTCTCGAGATGTTGGCACTAGTTGATCTGGATAAGCAGCCACTGTTTTG
TGCGGAACCCGAAAGATCTTTGCTATCCACAGGGCTTTGATAGTCAGATGTCTGTTCACA
TTCAAAAGGTGGGATCTGGAATGAAGCCAAAGCGAGGGCTGAGGCAGAGCCTTTCCGAAG
GACCTGTTGATAGATGTCTAGTAGGCAGTCCAGCTTGGATTCTATGGACTGTACCTGTTT
TTGAACCTTGACCACCCGACCGAGCATACTGAGATCGTCTGTGGTCTCATGTTCTGCTGT
TATTTTCTCTCGGCTCTTCTTATCTGATGTGATTTGCCCTTTTCCCTCGAGCCGAAT
```

sense primer

hKCNQ5 5'RACE primer. GSP1 GSP2 ~2157

5'RACE primer: 5' GCC AAA CAT GAG ACC ACA G 3' Tm=58° GSP1

5'RACE primer: 5' CTC GGT CGG GTG GTC AAG GTT 3' Tm=68° GSP2

5'RACE primer: 5' GGA CTG CCT ACT AGA CAT CTA TC 3' Tm=68 GSP2

hKCNQ5 Sense primer: 5' CTG GAT AAG CAG CCA CTG TTT 3' Tm=62°

1 of 1 3/9

DO NOT WRITE IN THIS MARGIN

: Design primers to use for 5'RACE: (see GIBCO's 5'RACE protocol)
Also one primer to use in PCR first — to see if hKCNQ5 is actually present in HEK 293 cells.

35

Created

SIGNED Joanne Trojnacki DATE

WITNESSED AND UNDERSTOOD BY: Priya Agarwal DATE

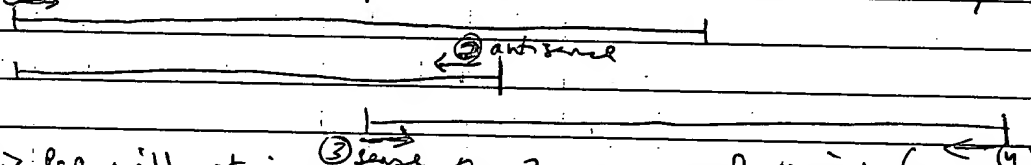
CROSS REFERENCES:

DATE: _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT hICCND5

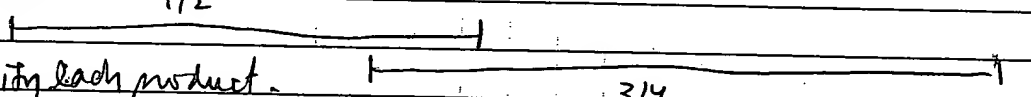
Cont'd. from p. 154

Design primers for PCR based on D5 Contig sequence: D5/#42, 61, 67, 47:
Use Marks overlapping PCR:
Do separate reactions (using plasmid DNA as template) - 1/2, 3/4



NOTE: This PCR will not incorporate the 7 amino acid insert (D5 isoform) of #42.

PCR products:



Gel purify each product.
Then use these 2 PCR products as template in one tube, using primers (1) and (4) to get a full length product. (~2100 bp)

* = most impt

D5 Contig Primers:

ID

5682 (1) Start w/ (sense) Some Kozak sequence GCC ACC ATG G and an EcoRV site to facilitate subcloning. - use #42 sequence

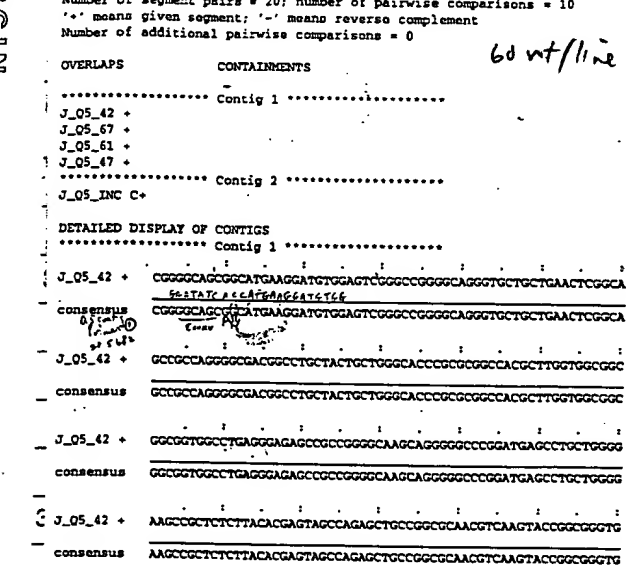
5683 (2) Antisense - common to all -

5684 (3) Sense - common to all

5685 (4) Stop w/ XbaI site to facilitate subcloning (antisense) - use #61/47 seq.

5686 (5) Stop w/ no restriction site built in (antisense) - use 61/47 seq

#64 incorrect orientation



```
Number of segment pairs = 20; number of pairwise comparisons = 10
'+' means given segment; '-' means reverse complement
Number of additional pairwise comparisons = 0

OVERLAPS          CONTAINMENTS                    60 nt/line

********** Contig 1 **********
J_Q5_42 +
J_Q5_67 +
J_Q5_61 +
J_Q5_47 +
********** Contig 2 **********
J_Q5_INC C+

DETAILED DISPLAY OF CONTIGS
********** Contig 1 **********

J_Q5_42 +  CGGGGCAGCGGCATGAAGGATGTGGAGTCGGGCCGGGGCAGGGTGCTGCTGAACTCGGCA
consensus  CGGGGCAGCGGCATGAAGGATGTGGAGTCGGGCCGGGGCAGGGTGCTGCTGAACTCGGCA

J_Q5_42 +  GCCGCCAGGGGCGACGGCCTGCTACTGCTGGGCACCCGCGCGGCCACGCTTGGTGGCGGC
consensus  GCCGCCAGGGGCGACGGCCTGCTACTGCTGGGCACCCGCGCGGCCACGCTTGGTGGCGGC

J_Q5_42 +  GGCGGTGGCCTGAGGGAGAGCCGCCGGGGCAAGCAGGGGGCCCGGATGAGCCTGCTGGGG
consensus  GGCGGTGGCCTGAGGGAGAGCCGCCGGGGCAAGCAGGGGGCCCGGATGAGCCTGCTGGGG

J_Q5_42 +  AAGCCGCTCTCTTACACGAGTAGCCAGAGCTGCCGGCGCAACGTCAAGTACCGGCGGGTG
consensus  AAGCCGCTCTCTTACACGAGTAGCCAGAGCTGCCGGCGCAACGTCAAGTACCGGCGGGTG

J_Q5_42 +  CAGAACTACCTGTACAACGTGCTGGAGAGACCCCGCGGCTGGGCGTTCATCTACCACGCT
consensus  CAGAACTACCTGTACAACGTGCTGGAGAGACCCCGCGGCTGGGCGTTCATCTACCACGCT

J_Q5_42 +  TTCGTTTTTCTCCTTGTCTTTGGTTGCTTGATTTTGTCAGTGTTTTCTACCATCCCTGAG
consensus  TTCGTTTTTCTCCTTGTCTTTGGTTGCTTGATTTTGTCAGTGTTTTCTACCATCCCTGAG

J_Q5_42 +  CACACAAAATTGGCCTCAAGTTGCCTCTTGATCCTGGAGTTCGTGATGATTGTCGTCTTT
consensus  CACACAAAATTGGCCTCAAGTTGCCTCTTGATCCTGGAGTTCGTGATGATTGTCGTCTTT

J_Q5_42 +  GGTTTGGAGTTCATCATTCGAATCTGGTCTGCGGGTTGCTGTTGTCGATATAGAGGATGG
consensus  GGTTTGGAGTTCATCATTCGAATCTGGTCTGCGGGTTGCTGTTGTCGATATAGAGGATGG

J_Q5_42 +  CAAGGAAGACTGAGGTTTGCTCGAAAGCCCTTCTGTGTTATAGATACCATTGTTCTTATC
consensus  CAAGGAAGACTGAGGTTTGCTCGAAAGCCCTTCTGTGTTATAGATACCATTGTTCTTATC
```

Cont'd →

SIGNED _____ DATE _____

CROSS REFERENCES: _____

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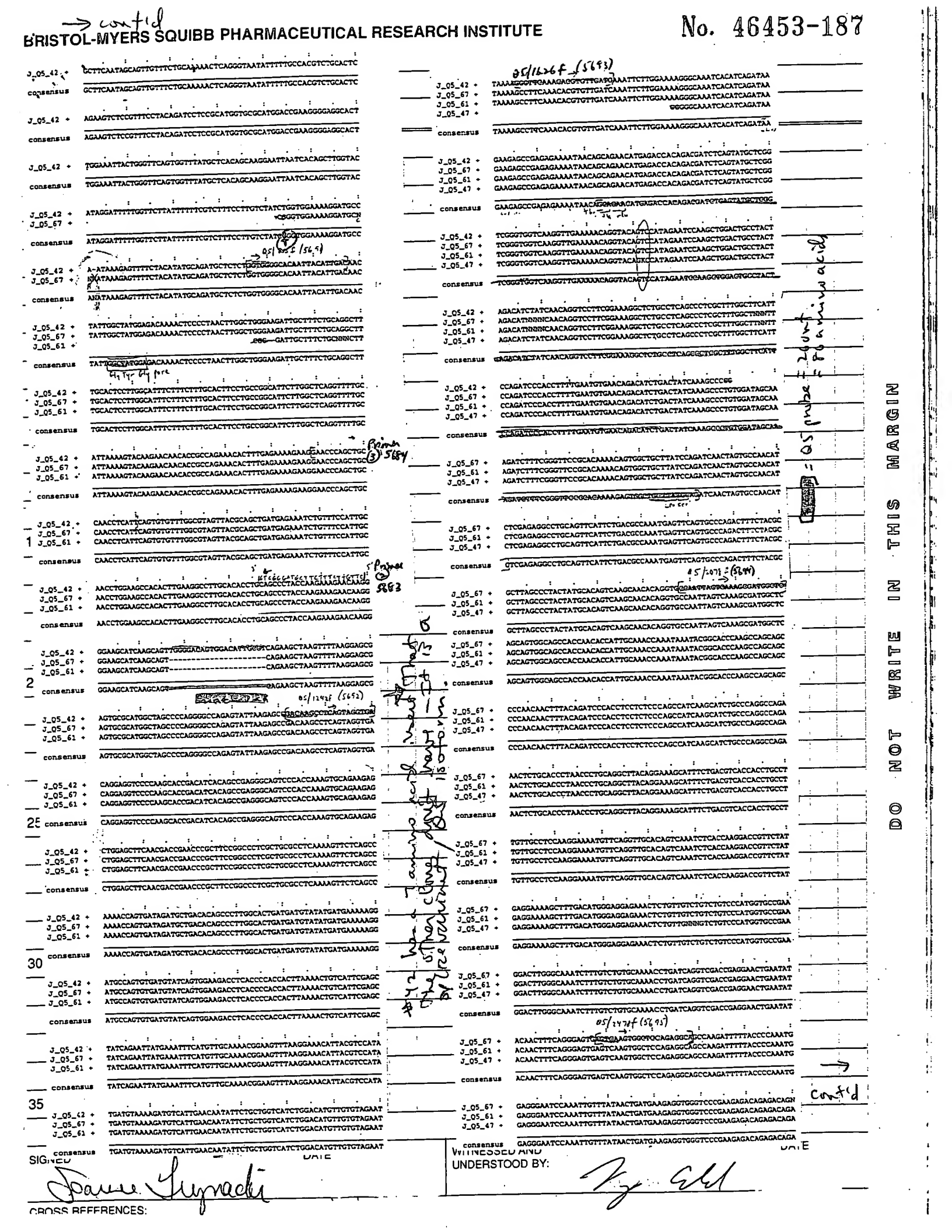
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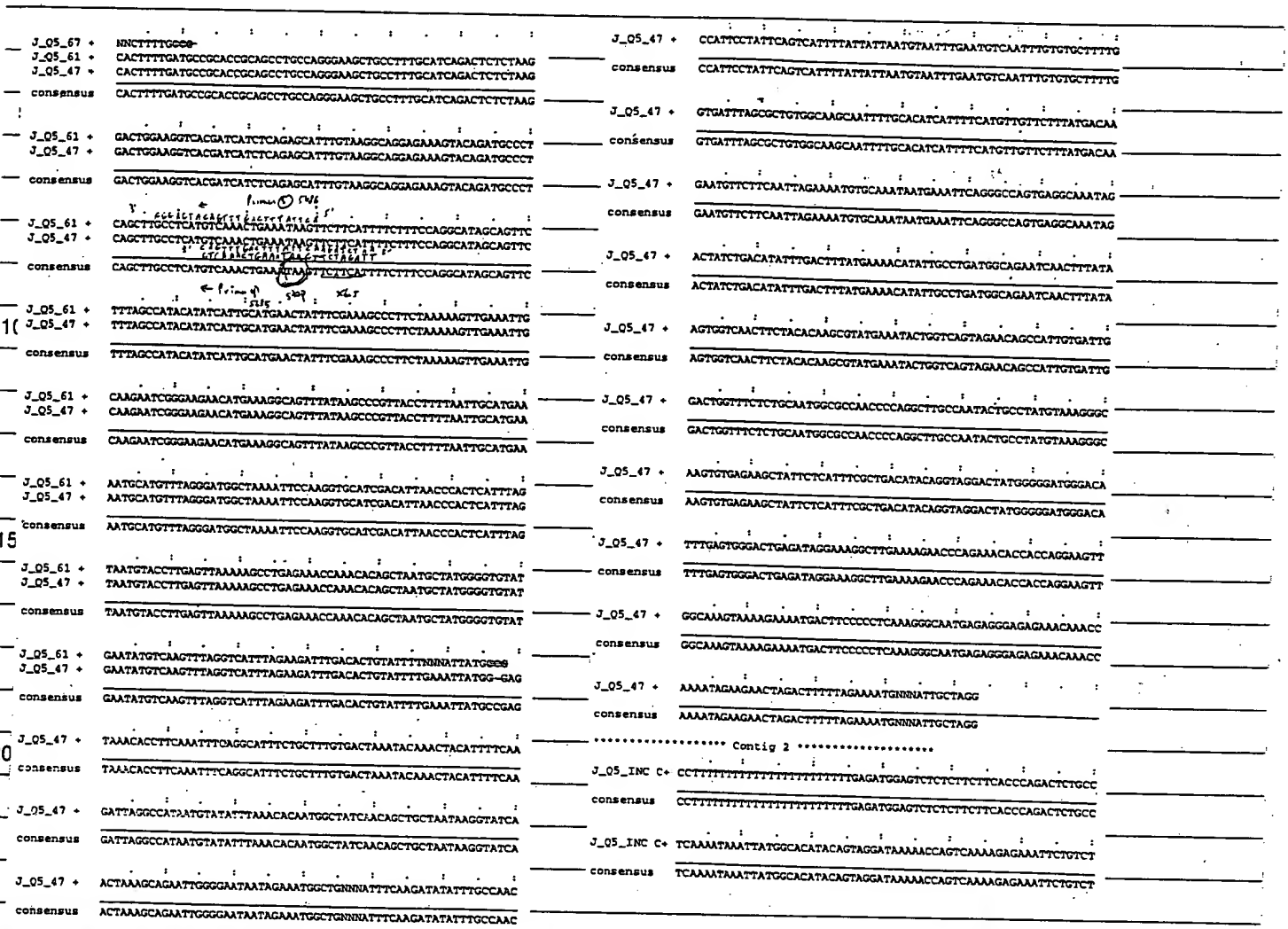
cont'd
SIGNED
DATE
WITNESSED AND UNDERSTOOD BY:
DATE
CROSS REFERENCES:

DATE: ___ PROJ. NO. ___ EXPT. NO. ___

SUBJECT hKcNQ5

→ cont'd



HHMI Biopolymer/Keck Foundation Biotechnology Resource Laboratory
Yale University School of Medicine

Oligonucleotide Synthesis Facility

Tm°

Inserted: **5682** - 5'-GGATATCACCATGAAGGATGTGG-3' 5' cont'g primer ① - Kpn I site/Eco R I ✓ — 68°
Inserted: **5683** - 5'-CTTGTTCTTTCTTGGTAGGGCTG-3' 5' cont'g primer ② - antisense — 68°
Inserted: **5684** - 5'-GAACCCAGCTGCCAACCTCATT-3' — 5' cont'g primer ③ (Sense) — 64°
Inserted: **5685** - 5'-AATCTAGAACTTATTTCAGTTTGAC-3' 5' cont'g primer ④ - Stop / Xba I primer — 66°
Inserted: **5686** - 5'-ACTTATTTCAGTTTGACATGAGGC-3' 5' cont'g primer primer - stop primer

THE ABOVE ORDERS HAVE BEEN SUCCESSFULLY PLACED!

Cont'd →

SIGNED Jamie Jurpiewicz DATE

WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DATE: PROJ. NO. EXPT. NO.

SUBJECT hkeNOS

Cont'd from p. 189

Template for PCR: Use Human Brain cDNA (#1, Clontech, Marathon-Ready) with the start/stop and start EcoRV/stop XbaI primers.
→ Tube (B) → Tube (E)

Also, use OS Plasmid DNA (#42, #64) for:

		Template
Tube (A)	start RV/(2) antisense	#42 plasmid DNA
Tube (B)	stop/(3) sense	#64 "
Tube (C)	stop XbaI/(3) sense	#64 "

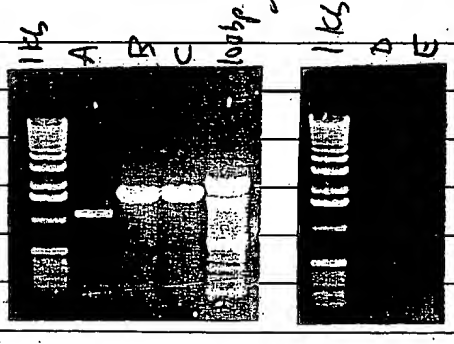
Dilute plasmid DNA (in pcDNA 3.1 + Neo) to 10 ng/μl – use #s –
#42 - use 2/11 Maxiprep at 2.82 μg/μl
#64 - use 2/18 Maxiprep at 2.11 μg/μl

Plasmid PCR:
#42 PCR Tubes (A) - 1140 bp to be amplified (at 1 min/kb) – extend at 68° for 1 min. 30 seconds
#64 PCR Tubes (B), (C) - 1720 bp to be amplified (at 1 min/kb) – extend at 68° for 2 minutes.

Human brain of
Hu Brain cDNA PCR:
Tubes (D), (E) - Start/stop primers: extend at 68° for 3 minutes.

94°, 3 minutes → 94° 30 sec → 55° 30 sec → 68° as indicated above + final 68°, 10 minutes. 25 cycles

Run 5% gel:



A - expect 1140 bp
B - expect 1720 bp
C - expect 1720 bp
D, E - expect 2700 bp

Cont'd →

SIGNED Dawn Gemmiti DATE

WITNESSED AND UNDERSTOOD BY: DATE

DATE: ___ PROJ. NO. ___ EXPT. NO. ___

SUBJECT hKcNQS

→ cont'd

For the Bmin full length : Tubes D = Kozak start EcoRV / stop

E = Kozak start EcoRV / stop XbaI

Have 2700 bp band — shotgun clone 3 µl into TA :

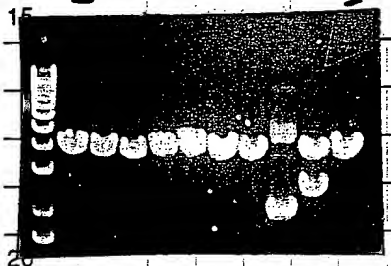
2 → vector, 1 µl 10x Ligase, 1 µl ligase, 3 µl H2O. Also do TA self-lig, 14° o.n.

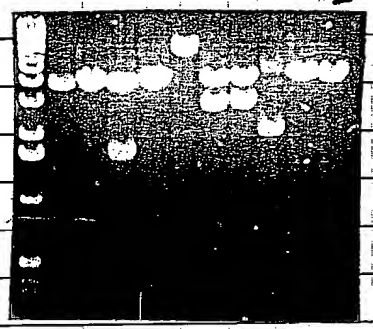
(NOTE - will need to prepare pcDNA 3.1(+)Neo + 5' RV/3'XbaI)

Transform 2 µl, plate out 10x + 100 µl onto 50 µg/ml Ctamp plate, 37° o.n.

	# Blue	# white	Set up minipreps :
TA - self-lig	70	5	
start/stop	200	25	#101-110
start/stop XbaI	200	25	#111-120

Wizard Plus Minipreps





OD, 1:50 :

260.0 S# 26

108 0.1688420 ng/µl
109 0.1693420 ng/µl
113 0.1801450 ng/µl
116 0.1826455 ng/µl
117 0.1830455 ng/µl
115 — Assume 400 ng/µl

Sequence :

Req'n #: 18914	CANCEL	Project:	HB (
For: trojnacj (Joanne Trojnacki)		Owner:	trojn
Status: Submitted		Comments:	Walli

8 reaction(s) to submit

No.	Template	Primer	Submitter's Comments	Operator' Comments
1.	HB_Q5_contig_PCR_no_109	M13rev		
2.	HB_Q5_contig_PCR_no_109	T7		
3.	HB_Q5_contig_PCR_no_115	M13rev		
4.	HB_Q5_contig_PCR_no_115	T7		
5.	HB_Q5_contig_PCR_no_116	M13rev		
6.	HB_Q5_contig_PCR_no_116	T7		
7.	HB_Q5_contig_PCR_no_117	M13rev		
8.	HB_Q5_contig_PCR_no_117	T7		

#109 - M13r - looks good - see start primer, Q5 sequence.

- T7 - weird peaks, but can see stop primer and Q5 sequence.

See sequencing results on p. 198

#116 - M13r : see stop primer w/ XbaI site, and Q5 sequence.

T7 - see start primer, RV site, Q5 seq. #116 is in the wrong orientation, in ?A, but will cut out Q5 w/ RV-built into start primer and XbaI built into stop primer and ligate Cont'd → pcDNA 3.1(+)Neo (RV/XbaI)

SIGNED Joanne Trojnacki DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: DATE

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DATE: PROJ. NO. EXPT. NO.

SUBJECT Hkcn05

→ cont'd (See page 192)

Also, have correct sized products for Tubes (A) - 5'stuf / (2) - #42
(B) (3) / 5'stp - #64
(C) (3) / 5'stp Xba - #64

Plasmid PCR -

Plasmid Templates

1140bp
1720bp
1720bp

Run Through Clontech's Nucleotrap PCR Purification - kit - Elute with 40µl H2O, for tube (A), 50x for tubes (B), (C); OD, 1:50;

	S#	260.0	280.0	λA/λB	S#	260.0	
In 40 → H2O	1 A	0.0028	0.0021	1.294	.28 µg total = 7 ng/λ		
50λ	2 B	0.0161	0.0097	1.666	2 µg total = 40 ng/λ		
50λ	3 C	0.0101	0.0056	1.804	1.26 µg total = 25 ng/λ		

Use in PCR: Templates A/B, A/C - with 5'stuf / 5'stp
dil. each 2 ng/λ - use 2.5λ or 5'stuf / 5'stp Xba primers

Use Plat. Taq Hi Fi

94°, 3 min.

94° 30" / 55° 30" / 68° 3' + 68°, 10' final
25 cycles

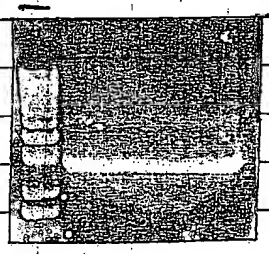
Run 5λ on gel:

	260.0	280.0	λA/λB	S#
	0.0224	0.0178	1.259	56 ng/λ

Looks good.
Ligate 2λ of each into TA vector.
Rest: clean up A/B of Nucleotrap PCR Purif. kit.
For tube A/C - cleanup w/ pcr purif. kit. Then digest w/ EcoRV / XbaI: eluted into 50λ H2O - save a few µl in case I need it - Rest:

DNA	10x REACT 2	10x BSA	H2O	Enz.
43λ	6	6	1	2 RV 2 XbaI, 37°, 2 hrs.

Run on gel:



Cut out - cleanup w/ clontech's Nucleotrap kit - OD: 1:50 ligate 2λ, 16°
pCDNA3 vector, 14° over weekend (actually was at 16°)
from 2/12/98 - 5' RV/3' XbaI at 30 ng/µl
3/6: Transform 2λ into DH5α competent cells, plate out 25λ, 250λ onto 100 µg/ml Lamp plates, 37° ovn.
~08 colonies
(25λ) self-lig ~ 2 colonies (250λ = 30 colonies) pick for minis - #121-140 Cont'd → Go top. 196 for minis

SIGNED Dawn Jezewski DATE

WITNESSED AND UNDERSTOOD BY: Joe Cahl DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

DATE: PROJ. NO. EXPT. NO.

SUBJECT hKEN#5

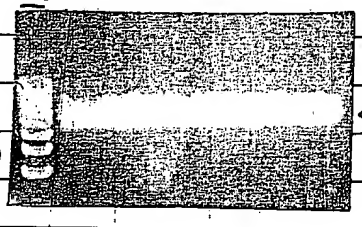
→ cont'd

Need to prepare some pcDNA3.1(+)Neo: 5'EcoRV/3'XbaI —

Have DNA from 2/26/99 at 1.19 µg/µl — Digest 5 µg:

DNA	NEB(2) 10X	BSA 10X	H$_2$O	Enz
4 µl	5	5	32	2 RV, 2 XbaI

37°, a few hrs. Run on gel:



Clean up Clontech's Nucleotrap kit - Elute 50 µl

H$_2$O, OD, 1:50:

260.0	280.0	λA/λB	S#	260
0.0228	0.0120	1.899	2.85 µg total in 50 µl = 57 ng/µl	

Use 1 µl in digestion w/ BS.

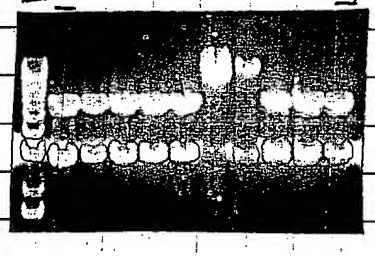
From A/c - RV/XbaI dig'd, from previous page - use 3 µl insert — 16° o.n.

3/8 - Transform 2 µl → DH5α comp. cells, plate out 25 µl, 250 µl each @ 100 µg/ml Ct amp plates, 37° o.n. Set up minipreps:

Self-lig (25 µl) - 16 colonies

Exp't'l (25 µl) - 31 colonies #141-150

Wizard Flex Mini, digest 2 µl w/ EcoRV/XbaI:



OD, 1:50:

#	260.0
#141	0.256640 ng/µl
142	0.145636 ng/µl
143	0.201984 ng/µl
146	0.186046 ng/µl

Sequence:

8 reaction(s) to submit

No.	Template	Primer	Submitter's Comments	Operator's Comments
1.	Q5_contig_PCR_2700bp_no_141	T7		
2.	Q5_contig_PCR_2700bp_no_141	BGHrev		
3.	Q5_contig_PCR_2700bp_no_142	T7		
4.	Q5_contig_PCR_2700bp_no_142	BGHrev		
5.	Q5_contig_PCR_2700bp_no_143	T7		
6.	Q5_contig_PCR_2700bp_no_143	BGHrev		
7.	Q5_contig_PCR_2700bp_no_146	T7		
8.	Q5_contig_PCR_2700bp_no_146	BGHrev		

See sequencing results in Book 47451, Page 24

Cont'd →

SIGNED Diane Leyneker DATE

WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

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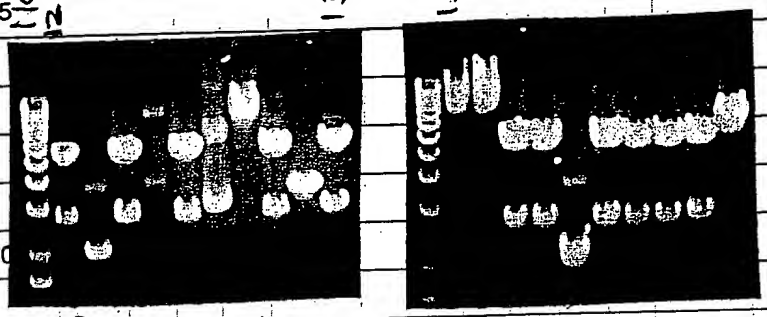
DATE: _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT hKCNB5

→ cont'd

Miniprep #124-140 (see p. 194) – Rxn A/C (Plasmid PCR) – 5'RV/XbaI dig'd, ligated into pCDNA3:

OD, 1:50!



3# 260.0

121 0.179548 ng/λ
132 0.200750 ng/λ
133 0.220255 ng/λ

Sequence:

Req'n #: 18933	CANCEL	Project:	Q5 Contig Plasmid
For:	trojnacj (Joanne Trojnacki)	Owner:	trojnacj (Joanne Troj
Status:	Submitted	Comments:	Wallingford

6 reaction(s) to submit

No.	Template	Primer	Submitter's Comments	Operator's Comments
1.	Q5_contig_PCR_2700bp_no_121	T7		
2.	Q5_contig_PCR_2700bp_no_121	BGHrev		
3.	Q5_contig_PCR_2700bp_no_132	T7		
4.	Q5_contig_PCR_2700bp_no_132	BGHrev		
5.	Q5_contig_PCR_2700bp_no_133	T7		
6.	Q5_contig_PCR_2700bp_no_133	BGHrev		

RESULTS:

Sequencing run # 17931, which contained samples from one or more requests you submitted, is now complete.

Below is the status of the items you requested. You can browse the CSF database for more information at: http://cyclops.hpw.pri.bms.com:8084/

*** PLEASE COME TO THE DNA SEQUENCING LAB NOW TO PICK UP YOUR SAMPLES ***

Project	Requ'n	Item	Template	Primer	Run ID	Lane	Status
Q5_Contig_Plasmid_PCR_2700bp_in_pcDNA3	18933	1	Q5_contig_PCR_2700bp_no_121	T7	17931	12	OK
Q5_Contig_Plasmid_PCR_2700bp_in_pcDNA3	18933	2	Q5_contig_PCR_2700bp_no_121	BGHrev	17931	15	Fail
Q5_Contig_Plasmid_PCR_2700bp_in_pcDNA3	18933	3	Q5_contig_PCR_2700bp_no_132	T7	17931	18	OK
Q5_Contig_Plasmid_PCR_2700bp_in_pcDNA3	18933	4	Q5_contig_PCR_2700bp_no_132	BGHrev	17931	21	OK
Q5_Contig_Plasmid_PCR_2700bp_in_pcDNA3	18933	5	Q5_contig_PCR_2700bp_no_133	T7	17931	24	OK
Q5_Contig_Plasmid_PCR_2700bp_in_pcDNA3	18933	6	Q5_contig_PCR_2700bp_no_133	BGHrev	17931	25	Fail

*** End of report ***

cont'd →

#132 - BGHrev: see XbaI site, stop site, Q5 sequence.
T7 - don't see start site - BLAST: next page.

#121 - BGHrev: failed
- T7: data a bit messy, but see start primer/start site and Q5 sequence.

SIGNED Joanne Trojnacki DATE

WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

DATE: PROJ. NO. EXPT. NO.

SUBJECT hKCNQ5

→ cont'd

Blast: #732 (See previous page):

'Q Family' Member

	Score (bits)	E Value
Sequences producing significant alignments:		
ref|NM_010611.1| Mus musculus potassium voltage-gated chan...	74	5e-11
ref|NM_004518.1| Homo sapiens potassium voltage-gated chan...	74	5e-11
gb|AF110020.1|AF110020 Homo sapiens potassium channel (KCNQ...	74	5e-11
gb|AF074247.1|AF074247 Homo sapiens neuronal delayed-rectif...	74	5e-11
gb|AF033348.1|AF033348 Homo sapiens potassium channel (KCNQ...	74	5e-11
emb|Y15065.1|HSKCNQ2 Homo sapiens mRNA for voltage gated po...	74	5e-11
dbj|D82346.1|D82346 Homo sapiens mRNA for HNSPC, complete cds	74	5e-11
dbj|AB000504.1|AB000504 Mus musculus mRNA for alternative s...	74	5e-11

ref|NM_010611.1|| Mus musculus potassium voltage-gated channel, subfamily Q, member 2 (Kcnq2), mRNA
Length = 2247

Score = 73.8 bits (37), Expect = 5e-11
Identities = 55/61 (90%)
Strand = Plus / Plus

```
Query: 51  tgcagaactacctgtacaacgtgctggagagaccccgcggctgggcgttcatctaccacg 110
           ||||||| | ||| |||||||||||| ||| | ||||||||||||||||||||||||||||
Sbjct: 316 tgcagaatttcctctacaacgtgctagagcggccccgcggctgggcgttcatctaccacg 375

Query: 111 c 111
           |
Sbjct: 376 c 376
```

ref|NM_004518.1|| Homo sapiens potassium voltage-gated channel, KQT-like subfamily, member 2 (KCNQ2) mRNA
Length = 7420

Score = 73.8 bits (37), Expect = 5e-11
Identities = 55/61 (90%)
Strand = Plus / Plus

```
Query: 51  tgcagaactacctgtacaacgtgctggagagaccccgcggctgggcgttcatctaccacg 110
           ||||||| | ||| ||||||||||||||| | || ||||||||||||||||||||||||||||
Sbjct: 272 tgcagaatttcctctacaacgtgctggagcggccgcgcggctgggcgttcatctaccacg 331

Query: 111 c 111
           |
Sbjct: 332 c 332
```

Score = 50.1 bits (25), Expect = 8e-04
Identities = 55/65 (84%)
Strand = Plus / Plus

```
Query: 449 cgcatggaccgaaggggaggcacttggaaattactgggttcagtggtttatgctcacagc 508
           |||||||||||| |||||||||| ||||| | ||||| || ||||| ||||| ||||||
Sbjct: 670 cgcatggaccggcggggaggcacctggaagctgctgggctctgtggtctatgcccacagc 729

Query: 509 aagga 513
           |||||
Sbjct: 730 aagga 734
```

Will pow up #121 for maxiprep → Qiagen Maxi - elute 200A TE, OD, 1:100

260.0	280.0	λA/λB	S#	260.0 (Nano ave)
0.6073	0.3232	1.879	759 µg/total	

= 3.03 µg/µl

Digest ~ 300 ng w/ EcoRV/XbaI, 37°, 1 hr.
Run on gel -

(Also made glycerol stock)

Cont'd →

SIGNED Joanne Yamadi DATE

WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

DATE: PROJ. NO. EXPT. NO.

SUBJECT hKCNQ5

→ cont'd (See p. 193)

I meantime, sequencing results of HBMS TA/Hu Brain, Q5 PCR prod (2700 bp), #116:

In TA

HB_Q5_contig_PCR_no_116_T7.1791
[Strand]

EcoRI EcoRV start

1 GAATTCGGCT TGGATATCAC CATGAAGGAT GTGGAGTCGG GCCGGGGCAG GGTGCTGCTG AACTCGGCAG CCGCCAGGGG

GluPheGlyLeu AspIleThr METLysAsp ValGluSerGly ArgGlyArg ValLeuLeu AsnSerAlaAla AlaArgGl

81 CGACGGCCTG CTACTGCTGG GCACCCGCGC GGCCACGCTT GGTGGCGGCG GCGGTGGCCT GAGGGAGAGC CGCCGGGGCA

yAspGlyLeu LeuLeuLeuGly ThrArgAla AlaThrLeu GlyGlyGlyGly GlyGlyLeu ArgGluSer ArgArgGlyL

161 AGCAGGGGGC CCGGATGAGC CTGCTGGGGA AGCCGCTCTC TTACACGAGT AGCCAGAGCT GCCGGCGCAA CGTCAAGTAC

ysGlnGlyAla ArgMETSer LeuLeuGlyLys ProLeuSer TyrThrSer SerGlnSerCys ArgArgAsn ValLysTyr

241 CGGCGGGTGC AGAACTACCT GTACAACGTG CTGGAGAGAC CCCGCGGCTG GGCGTTCATC TACCACGCTT TCGTTTTTCT

ArgArgValGln AsnTyrLeu TyrAsnVal LeuGluArgPro ArgGlyTrp AlaPheIle TyrHisAlaPhe ValPheLe

321 CCTTGTCTTT GGTTGCTTGA TTTTGTCAGT GTTTTCTACC ATCCCTGAGC ACACAAAATT GGCCTCAAGT TGCCTCTTGA

uLeuValPhe GlyCysLeuIle LeuSerVal PheSerThr IleProGluHis ThrLysLeu AlaSerSer CysLeuLeuI

401 TCCTGGAGTT CGTGATGATT GTCGTCTTTG GTTTGGAGTT CATCATTCGA ATCTGGTCTG CGGGTTGCTG TTGTCGATAT

leLeuGluPhe ValMETIle ValValPheGly LeuGluPhe IleIleArg IleTrpSerAla GlyCysCys CysArgTyr

481 AGAGGATGGC AAGGAAGACT GAGGTTTGCT CGAAAGCCCT TCTGTGTTAT AGATACCATT GTTCTTATCG CTTCAATAGC

ArgGlyTrpGln GlyArgLeu ArgPheAla ArgLysProPhe CysValIle AspThrIle ValLeuIleAla SerIleAl

561 AGTTGTTTCT GCAAAAACTC AGGGTAATAT TTTTGCCACG TCTGCACTCA GAAGTCTCCG TTTCCTACAG A

aValValSer AlaLysThrGln GlyAsnIle PheAlaThr SerAlaLeuArg SerLeuArg PheLeuGln

Has open reading frame.

HB_Q5_contig_PCR_no_116_M13rev.
[Strand]

EcoRI XbaI stop

1 GAATTCGGCT TAATCTAGAA CTTATTTCAG TTTGACATGA GGCAAGCTGA GGGCATCTGT ACTTTCTCCT GCCTTACAAA

81 TGCTCTGAGA TGATCGTGAC CTTCCAGTCC TTAGAGAGTC TGATGCAAAG GCAGCTTCCC TGGCAGGCTG CGGTGCGGCA

161 TCAAAAGTGT CTGTCTCTGT CTCTTCGGGA CCCACCTCTT CATCAGTTAT AAACAATTTG GATTCCCTCC ATTTGGGGTA

241 AAAATCTTGG CTGCCTCTGG AGCCACTTGA CTCACTCCCT GAAAGTTGTA TATTCAGTTC CTCGGTCGAC CTGATCAGGT

321 TTTGCACAGA CAAAGATTTG CCCAAGTCCT TCGGCACCAT GGGACAGACA GACAACAGAG TTTCTCCTCC CATGTCAAAG

401 CTTTTCCTCA TAGAACGGTC CTTGGTGAGA TTTGACTGTG CAACCTGACA TTT

#116, inTA- in wrong orientation but can cut it with EcoRV at the 5' end of insert, and XbaI at the 3' end of the insert. These sites were designed into the primers used in PCR:

Cont'd →

SIGNED Joanne Szpiech DATE

WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

DATE PROJ. NO. EXPT. NO.

SUBJECT hKCNQ5

→ cont'd

In TA

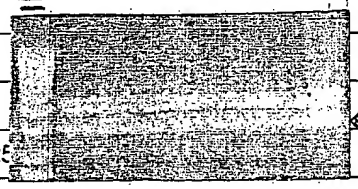
HB_Q5_contig_PCR_no_116_M13rev. Rev'd/comp'l'd
[Strand]

```
1    AAATGTCAGG TTGCACAGTC AAATCTCACC AAGGACCGTT CTATGAGGAA AAGCTTTGAC ATGGGAGGAG AAACTCTGTT
     LysCysGlnVal AlaGlnSer AsnLeuThr LysAspArgSer METArgLys SerPheAsp METGlyGlyGlu ThrLeuLe
81   GTCTGTCTGT CCCATGGTGC CGAAGGACTT GGGCAAATCT TTGTCTGTGC AAAACCTGAT CAGGTCGACC GAGGAACTGA
     uSerValCys ProMETValPro LysAspLeu GlyLysSer LeuSerValGln AsnLeuIle ArgSerThr GluGluLeuA
161  ATATACAACT TTCAGGGAGT GAGTCAAGTG GCTCCAGAGG CAGCCAAGAT TTTTACCCCA AATGGAGGGA ATCCAAATTG
     snIleGlnLeu SerGlySer GluSerSerGly SerArgGly SerGlnAsp PheTyrProLys TrpArgGlu SerLysLeu
241  TTTATAACTG ATGAAGAGGT GGGTCCCGAA GAGACAGAGA CAGACACTTT TGATGCCGCA CCGCAGCCTG CCAGGGAAGC
     PheIleThrAsp GluGluVal GlyProGlu GluThrGluThr AspThrPhe AspAlaAla ProGlnProAla ArgGluAl
321  TGCCTTTGCA TCAGACTCTC TAAGGACTGG AAGGTCACGA TCATCTCAGA GCATTTGTAA GGCAGGAGAA AGTACAGATG
     aAlaPheAla SerAspSerLeu ArgThrGly ArgSerArg SerSerGlnSer IleCysLys AlaGlyGlu SerThrAspA
401  CCCTCAGCTT GCCTCATGTC AAACTGAAAT AAGTCTAGA TTAAGCCGAA TTC
                                    XbaI                  EcoRI
     laLeuSerLeu ProHisVal LysLeuLysSTP ValLeuAsp STPAlaGlu Phe
```

Has open reading frame up to the stop site.

Digest ~5 µg of cClone #116 (HB Brain):

	DNA	10× NEB(2)	10× BSA	H_2O	Enz
20	10λ	5	5	26	2 EcoRV, 2 XbaI, 37°, 2 hrs. Run on gel:



← 2.7 kb (~2 µg of insert)

Cut out indicated band. Clean up w/ Clontech Nucleotrap kit. Elute w/ H2O (50λ) – use 3λ to ligate into pcDNA3.1(+)Neo (5'EcoRV/3'XbaI) – 16° o.n.

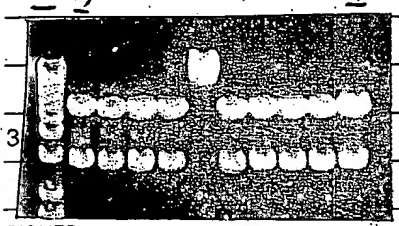
3/9: Transform 2λ into DH5α competent cells. Plate out 25λ, 50λ onto 100µg/ml Lamp plates, 37° o.n.

Set up minipreps:

RESULTS: Self-lig (25λ) ~ 1000 colonies

Expt'l (25λ) ~ 250 colonies #151-160

Wizard Plus. Digest 2λ w/ EcoRV/XbaI, run on gel:



OD, 1:50:

260.0

155 0.1532 380 µg/λ
156 0.1658 415 µg/λ
157 0.1356 340 µg/λ

S#. For sequencing results see Book 47451, page 24

Cont'd →

SIGNED Joanne Trzaskos DATE

WITNESSED AND UNDERSTOOD BY: Tyra Gold DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE: _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT hKCNQ5

→ santa (See Q5 sequence on pg. 186-188 for details)

Design primers for sequencing entire Q5. → primers highlighted in sequence.

HHMI Biopolymer/Keck Foundation Biotechnology Resource Laboratory
Yale University School of Medicine

Oligonucleotide Synthesis Facility

OLIGO ORDER DETAILS

DO NOT WRITE IN THIS MARGIN

1
Order Date:
Sequence Name: 5690 Q5/396f
Length: 22mer
Scale: 40.0
Sequence: 5'-GGAGTTCGTGATGATTGTCGTC-3'
Notes:
Synthesis Date: Chargaff

Order Date:
Sequence Name: 5691 Q5/875f
Length: 22mer
Scale: 40.0
Sequence: 5'-GGTGGGGCACAATTACATTGAC-3'
Notes:
Synthesis Date: Chargaff

2
Order Date:
Sequence Name: 5692 Q5/1242f
Length: 19mer
Scale: 40.0
Sequence: 5'-GACAAGCCTCAGTAGGTGA-3'
Notes:
Synthesis Date: Chargaff

Order Date:
Sequence Name: 5693 Q5/1626f
Length: 21mer
Scale: 40.0
Sequence: 5'-GCCTTCAAACACGTGTTGATC-3'
Notes:
Synthesis Date: Chargaff

3

Order Date:
Sequence Name: 5694 Q5/2078f
Length: 23mer
Scale: 40.0
Sequence: 5'-CCAATTAGTCAAAGCGATGGCTC-3'
Notes:
Synthesis Date: Chargaff

Order Date:
Sequence Name: 5695 Q5/2478f
Length: 21mer
Scale: 40.0
Sequence: 5'-AGTCAAGTGGCTCCAGAGGCA-3'
Notes:
Synthesis Date: Chargaff

35

Continued in Book 47451, page 21

SIGNED Laurie Gyuradi DATE

WITNESSED AND UNDERSTOOD BY: Yge Glel DATE

CROSS REFERENCES: